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A Microfluidic Chip Integrated with Droplet Generating, Pairing, Trapping, Merging, Mixing and Releasing

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S1 Optimization of the design

The first version of the design is shown in Figure S1 where the overview of the entire channel network is shown in Figure S1A, the droplet generator Figure S1B, the oil regulator for adjusting droplet spacing Figure S1C and the droplet trapper Figure S1D. The droplet pair generator is adopted from the design proposed by Frenz *et al* [14]. The advantage of this generator is that the two T-junctions share one continuous phase channel, thus reduces one inlet and simplifies the flow circuit. However, this droplet generator cannot control the size of the droplets carried in the two streams independently, that is one droplet stream affects the other. For example, when a droplet from either of the two steams (for example, one droplet with reagent #1) enters the front of the trapping well, it has two choices, either entering the trapping well or the bypass channel. The bypass channel is designed to have a higher resistance than that of the trapping well (the bypass channel length is set at 3200 μm based on some preliminary experimental testing). Therefore, the droplet with reagent I will enter the trapping well and increase the resistance of the trapping well dramatically so that the following droplets will go through the bypass channel. The trapped droplet can stay inside the trapping well due to interfacial tension and wait for a droplet with reagent II getting trapped. The bypass channel and trapping well for reagent II have the same dimensions as that for reagent I. Therefore, when a droplet with reagent II reaches the front of the trapping well, it will get trapped. The trapping wells for droplet 1 and droplet 2 intersect with a gap so that the trapped droplets can merge. The following droplets will go through bypass channel and follow the same rule in the downstream of the trapping wells.

Preliminary tests were performed to examine each function. The results show that this generator can generate two streams of droplets and the trapping wells can trap and merge two droplets from two streams as anticipated. However, some drawbacks associated this design result in low robustness of the system performance and briefly, the two T-junction droplet generators affect each other because they share one oil channel which does not allow the droplet size from each stream to be controlled independently. The design does not allow droplets to be generated on demand. Consequently, if droplet generation is not stable which often happens at the beginning, droplets with undesired size will be trapped which prevents desired droplets to be trapped later. It is also difficult to reuse the chip for multiple assays as there is no chemical waste for releasing droplets after reaction. It is also prone to dust that could come into the channels as shown in Figure S2.

The second version of the design was proposed to address the above drawbacks associated with the first version, as shown in Figure S3. In this design, the droplet generator can generate droplets containing reagent I and II alternatively [30] into one channel instead of two in the first design, which also reduces the two bypass channels into one. The simplified design will reduce the challenge in synchronizing two streams of droplets and increase the robustness of the system performance. This design also allows

droplets to be generated on-demand by tuning the applied pressure at the inlets such that non-uniform droplets that are generated before the system is stabilized are pumped into the waste reservoir without being trapped. In addition, a channel branch is added to dispose chemical waste with a particular consideration of minimizing the flow fluctuation when tuning the applied pressures by designing the waste channel to have the same dimension as that of the trapping channel. In order to ensure the droplet trapping works robustly, the resistance of the bypass channel is designed to be larger than that of the trapping wells without droplets so that droplets will preferably go into the traps, but smaller than that of the trapping wells with droplets trapped so that the rest of coming droplets will go through the bypass channel. The same situation will occur for all the trapping wells. As a result, the number of droplets trapped in each well is controllable. In order to reuse the chip for multiple assays without interruption, this design adds a waste channel through which the reaction waste can be pumped into the waste reservoir by tuning the applied pressures. For example, a combination of high pressure applied in outlet 1 and no pressure in outlet 2 would flush the chemical waste in the trapping wells into the waste reservoir (outlet 2) through the waste channel.

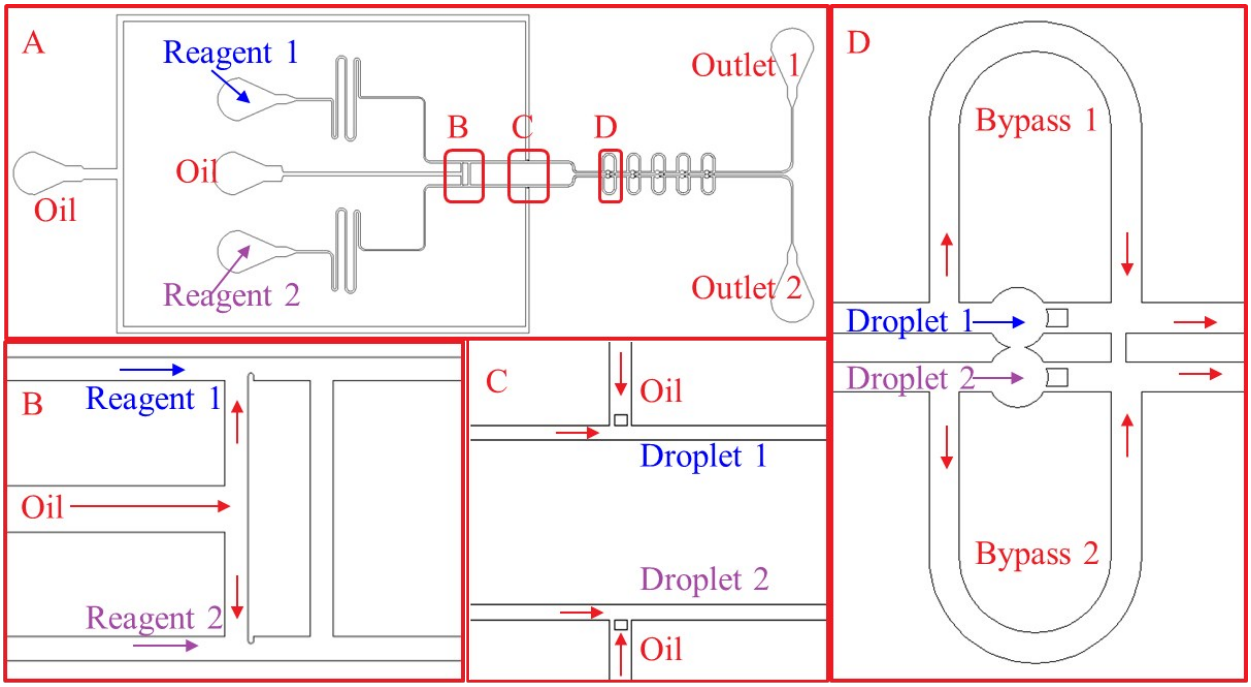

Figure S1 Sketch of the first version design for trapping two streams of droplets. A) Overview of the chip design. B) Droplet generator to generate two streams of droplets with channel height of 60 μm , channel width of oil 240 μm , channel widths of 120 μm for both reagent #1 and #2. C) A regulator to adjust the spacing between two droplets. D) Trapping well to trap two droplets from two different streams. An array of trapping wells can be integrated into the flow stream depending on requirements. Five trapping wells are integrated in this design to demonstrate the working principle.

Some preliminary experiments were performed to test the design for its trapping and merging performance as shown in Figure S4. Although the trapping wells can successfully trap and merge droplets and this design has a much simpler structure, its robustness is still not as good as expected for practical applications due to the following reasons:

- It is difficult to adjust the flow rates of each fluid to ensure an alternative droplet generation with 1:1 ratio.

- The droplet generation mode is sensitive to the pressure fluctuation downstream. When switching the pressures in outlet 1 and outlet 2, a small flow fluctuation could break the droplet generation rule (from 1:1 ratio to other ratios).
- When some dust (e.g. a small piece of PDMS) occasionally goes into the channel, it is difficult to remove it to the sample waste channel due to the pillar in the waste channel inlet. Nevertheless, the pillar should be kept there in order to prevent droplets going inside the waste channel when droplet trapping is triggered.

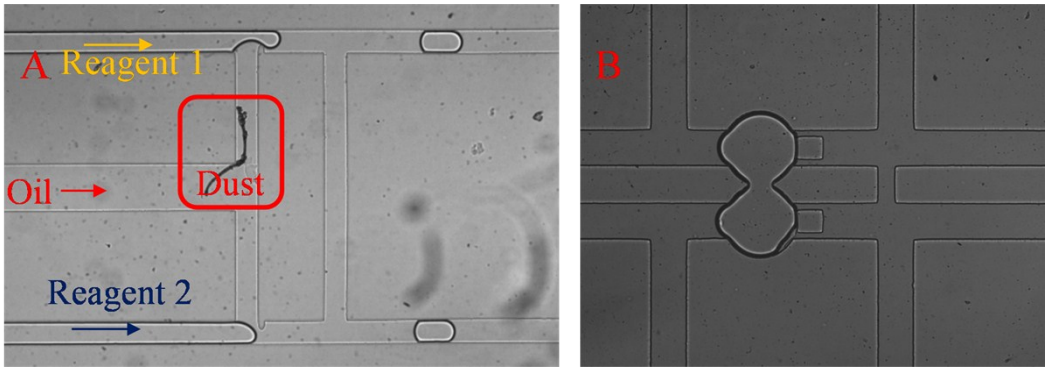

Figure S2 A. Picture of droplet generation captured using $4\times$ magnification. B. Picture of trapped droplets captured using $10\times$ magnification.

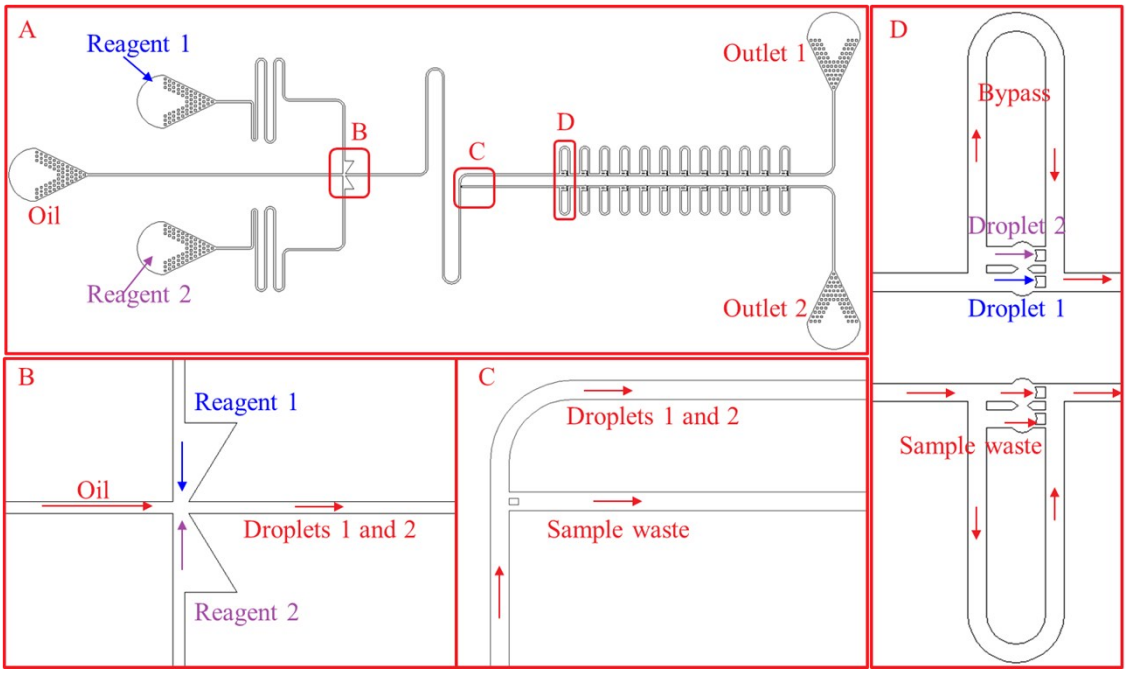

Figure S3 Sketch of the second version design for trapping two streams of droplets. A) Overview of the chip design. B) Droplet generator for generating two streams of droplets alternatively with channel height and width $60\,\mu m$ and $120\,\mu m$, respectively. The reagents channels have a triangle pressure oscillator. C) A channel with a rectangular pillar at the entrance is added beside the trapping channel to dispose sample waste and both channels have the same dimensions. D) Trapping wells to trap two droplets from upstream.

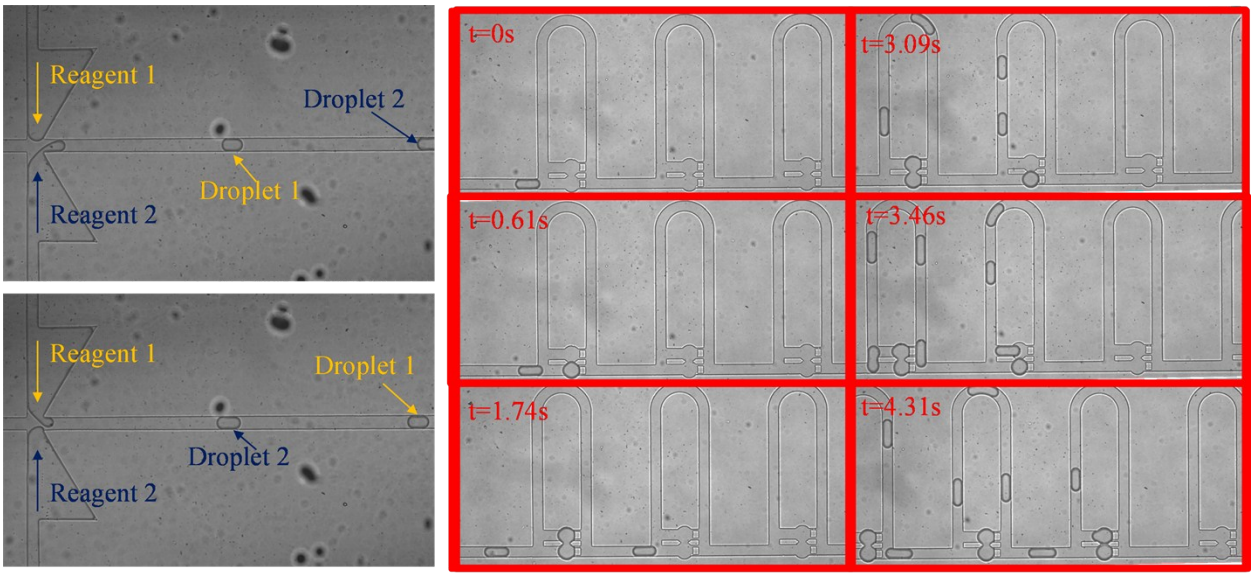

Figure S4 (A) Images captured using 4× magnification show droplets that are generated alternatively. B. Images show the droplet trapping process captured using 4× magnification.

S2 Determination of the final concentration of the merged droplet

The final concentration of methylene blue is better quantified by the volume ratio of two droplets before merging than by gray scale values which is more influenced by imaging processing. The final concentration of methylene blue can be calculated as,

$$C_{final} = \frac{V_{d\_dye}}{V_{d\_dye} + V_{d\_water}} \times C_0 \qquad \text{(S1)}$$

where C_{final} is the final concentration of methylene blue after merging and mixing, C_0 the original concentration of methylene blue in the droplet before merging, V_{d_water} the volume of the droplet containing pure water before merging, and V_{d_dye} the volume of methylene blue droplet before merging. Droplet volume can be measured by image processing or calculated by $V_d = Q_d/f$, where V_d is the droplet volume, Q_d the flow rate of the dispersed phase, and f droplet generation frequency.

S3 Experimental measurement of fluorescence for drug screening reactions

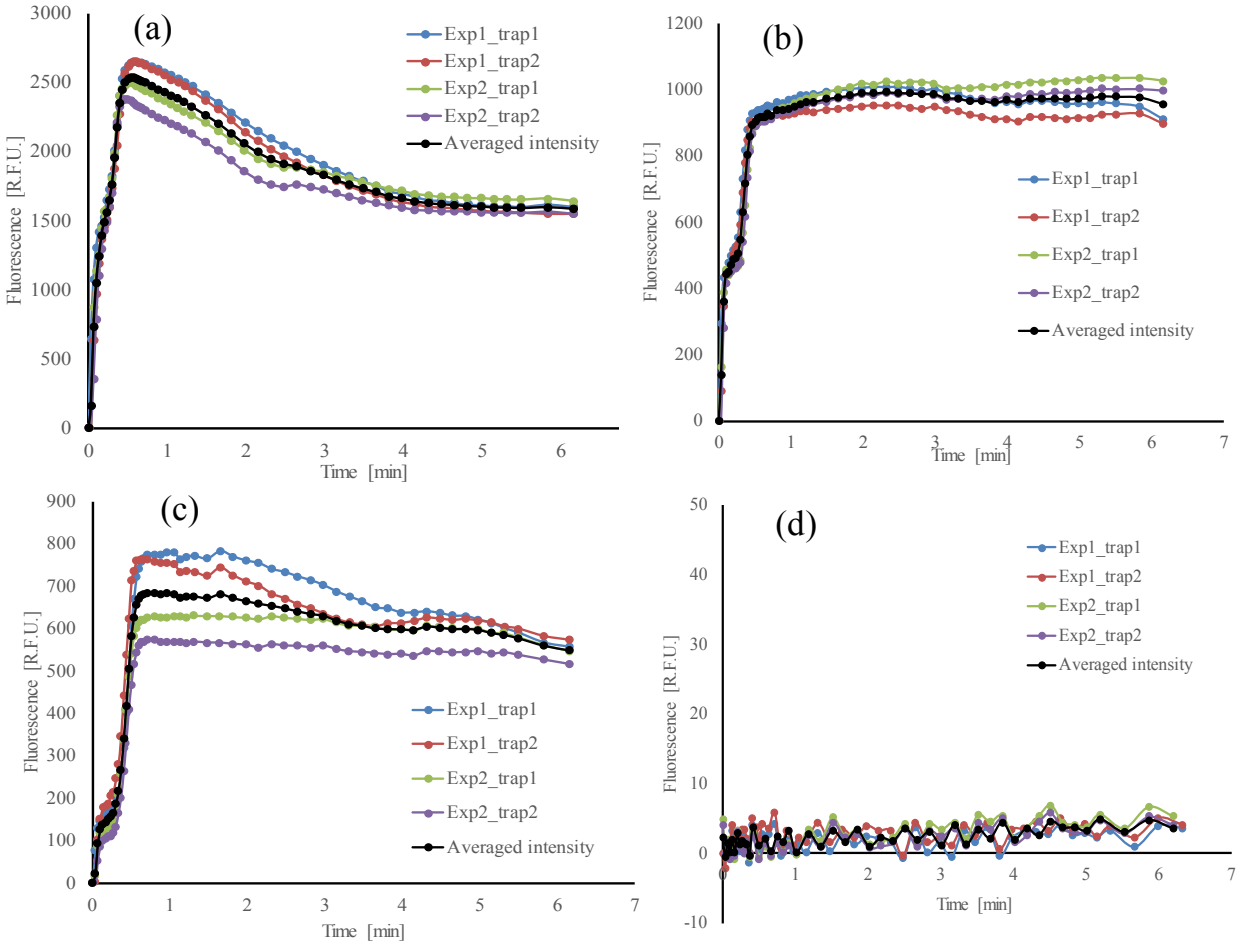

Figure S5 Plots of Fluorescence intensity over time. The intensity data subtract from the background intensity (the first frame before droplet merging) for each experiment. (a) Positive control (AcPHF6); (b) AcPHF6 + 16.5μM Orange G; (c) AcPHF6 + 33μM Orange G; (d) Negative control (ThT). Each experiment was duplicated and the intensity data was captured from two trapping wells simultaneously during one experiment.